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Case report

A suicidal poisoning due to tramadol. A metabolic approach to death investigation

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ABSTRACT

Tramadol is a synthetic opioid, widely used for post-surgical and chronic pain. Lethal overdose due only to tramadol is not common; more often the poisoning is due to tramadol in combination with other substances.

Reported is a suicidal case of lethal tramadol poisoning in a 48-year-old woman. Tramadol and its metabolites O-desmethyltramadol (M1), N-desmethyltramadol (M2), N,N-didesmethyltramadol (M3), N,O-didesmethyltramadol (M5) were detected by GC/MS in biological fluids (femoral blood, bile, urine, gastric content) and viscera (brain, lung, liver and kidney). The tramadol concentration in femoral blood was 61.83 mcg/ml which is approximately 30 times higher than that believed to be lethal.

According with other Authors, a preferential formation of M1 over M2 (M1/M2 ratio >1) is indicative of acute death, while M1/M2 ratio <1 suggests that death occurred after a longer time lapse from ingestion.

1. Introduction

Tramadol is a centrally acting synthetic opioid.[1,2] It is commonly used in clinical practice as analgesic, both for patients in the post-surgical period and also in pain therapy for chronic pain syndromes. Although its exact mechanism of action is not yet completely understood, it is well known that tramadol possesses weak agonist actions at the mu-opioid receptor (binding of parent and the O-demethylated (M1) metabolite to mu-opioid receptors).[1] It has been shown that tramadol blocks serotonin and norepinephrine reuptake.[1] However, like other narcotics used for the treatment of pain, tramadol is often abused.[3]

Tramadol is available in many forms as capsules, tablets for sublingual and oral routes, suppositories, solution for subcutaneous, intramuscular, and intravenous injection, and solutions for injection by the various spinal routes (epidural, intrathecal, caudal and others).

The pharmacokinetics of tramadol has been extensively described.[4,5] It is well absorbed after oral administration (approximately 75%) and the mean peak plasma concentration of racemic tramadol and M1 occurs at two and three hours, respectively.[6] Therapeutic concentrations are in a range between 0.1 and 0.8 mcg/ml; toxic effects may occur at blood concentrations above 1 mcg/ml. Deaths related to tramadol intoxications have been reported at 2 mcg/ml or higher.[6] M1 metabolite is also believed to cause some toxic effects of the drug.[7]

After oral administration tramadol is metabolized by a number of pathways, including CYP2D6 and CYP3A4, as well as by conjugation of parent and metabolites. The human *in vivo* metabolism of tramadol is complex with 23 metabolites identified: 11 phase I metabolites and 12 phase II conjugates.[4] O-desmethyltramadol (M1), N-desmethyltramadol (M2), N,N-didesmethyltramadol (M3) and N,O-didesmethyltramadol (M5) are reported as major phase I metabolites.[4] Tramadol's metabolites are primarily excreted through kidneys (almost 29% as unchanged drug).

Despite this substance being widely used, an acute lethal poisoning of the drug is a rare occurrence as there are only 15 fatal intoxications cited in the Literature.[7,8] Usually poisoning induced

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by tramadol is reported in combination with other substances (alcohol, benzodiazepines, barbiturates, antidepressants, etc.).[9–17]

Illustrated is a suicide case of lethal tramadol poisoning, in which were found the highest concentrations of tramadol described so far. The concentrations of tramadol and its major metabolites found in all biological specimens are also reported.

2. Case history

A 48-year-old Caucasian woman was found dead in her home. The woman's medical history showed that she suffered from mental disorders and was hospitalized several times in psychiatric facilities. In 2005 she had already attempted suicide. Beside the corpse there were no boxes of medicine. Her doctor stated that the woman, for many years, was treated with different drugs including carbamazepine and tramadol.

Autopsy revealed no signs of criminal activity, nor visible pathological processes related to the death.

The histological examination showed polivisceral congestion. In particular were evidenced the presence of thrombi and blood clots in the chambers of the heart and partly hemorrhagic pulmonary edema, vicarious acute emphysema, very large amount of hemosiderophagic histiocytes and focal areas of endoalveolar hemorrhage, signs of low blood flow. No associated pathologies were observed.

3. Materials and methods

3.1. Samples

Systematic toxicological analysis was preliminarily performed on femoral blood, urine and gastric content samples to investigate for illegal and prescribed drugs, alcohol, volatile substances and other poisons. These preliminary tests indicated the presence of tramadol and its metabolites, and of carbamazepine. Tramadol and its metabolites quantification were performed on viscera (brain, lung, liver and kidney) and biological fluids (femoral blood, bile, urine, gastric content). Carbamazepine quantification was performed on femoral blood.

3.2. Reagents

Diethyl ether, 1-Chlorobutane, water, n-hexane and n-butyl acetate were all SupraSolv for gas chromatography. Sulfuric acid, concentrated ammonium hydroxide and all the other solvents used were purchased from Merck KGaA (Darmstadt, Germany).

3.3. Standards

Working standards of pharmaceutical grade tramadol hydrochloride as well as O-desmethyltramadol (M1), N-desmethyltramadol (M2), N,N-didesmethyltramadol (M3) and N,O-didesmethyltramadol (M5) were obtained as generous gifts from Grünenthal GmbH (Aachen; Germany). Of each substance, a standard stock solution of 1 mg/ml was prepared in methanol; then, serial dilutions were made to get the working standard solutions.

3.4. Toxicological analysis on viscera and biological fluids

The toxicological analyses for tramadol and its metabolites quantification were performed on 2 g of viscera (brain, lung, liver and kidney) and on 2 ml of biological fluids (femoral blood, bile, urine, gastric content) using the method suggested by Bynum et al.[15] suitably modified. Scopolamine (50 mcg/ml or mcg/g) were used as internal standard.

The samples were analyzed using two different GC/MS acquisition methods: by full scan acquisition method (SCAN) to resolve tramadol, M2, M3 and hydroxytramadol and by select ion monitoring (SIM) to resolve the derivatized M1 and M5.

For the SCAN analysis, each dry extract was reconstituted in 200 mcl of acetone and directly analyzed by GC/MS.

For the SIM analysis aliquot parts of the samples were then evaporated to dryness and derivatized with 50 mcl of N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) and heated at 70 °C for 30 min; the analysis was performed by monitoring the following ions: *m/z 58; 303; 393* (M1-TMS); *m/z 116; 246; 451* (M5-TMS); *m/z 138; 154; 375* (scopolamine TMS).

The gas chromatography–mass spectrometry (GC–MS) technique used in this study for carbamazepine quantification on blood specimens is carried out according to the procedure recommended by Speed et al.[18]

3.5. Apparatus

The GC/MS analysis was carried out using an Agilent Technologies (AT) model 6890N GC coupled with an AT mod. 5973 Inert mass selective detector and an AT 7683 Series automatic sampler.

An EVDX-5MS (Agilent) crosslinked fused-silica capillary column (25 m × 0.20 mm i.d. × 0.33 mcm film thickness) was linked to the mass selective detector (MSD) through a direct capillary interface. The injector and interface temperatures were 280 °C.

The oven temperature was maintained at 100 °C for 2 min, then programmed to 250 °C at 30 °C/min, to 300 °C at 5 °C/min, and maintained at 300 °C for 3 min. Source temperature was 230 °C, and quadrupole temperature was 150 °C. The carrier gas was helium with a flow rate of 1.5 ml/min.

3.6. Calibration curves

The method applied is characterized by a limit of detection (LOD) for tramadol and M1 of 10 ng/ml and by a limit of quantitation (LOQ) of 40 ng/ml.

However, taking into consideration the high concentrations to be determined in the case reported, the calibration curves were constructed by analyzing control samples added with tramadol (50–1000 mcg/ml); O-desmethyltramadol (5–100 mcg/ml); N-desmethyltramadol (50–1000 mcg/ml); N,N-didesmethyltramadol (5–100 mcg/ml); N,O-didesmethyltramadol (5–100 mcg/ml) and using as internal standard scopolamine (50 mcg/ml or mcg/g).

4. Results

Tramadol and its metabolites M1, M2, M3, M5 were detected in all samples analyzed; their concentrations (mcg/ml or mcg/g) are shown in Table 1.

Table 1
Concentrations of tramadol and its major metabolites detected in viscera (mcg/g) and biological liquids (mcg/ml).

Specimen	T	M1	M2	M3	M5
Blood (femoral)	61.83	14.13	134.25	23.61	3.71
Brain	44.21	7.86	90.43	9.72	1.49
Lung	106.06	18.24	181.20	30.35	5.31
Liver	56.19	16.21	148.24	28.06	7.90
Kidney	88.04	23.01	161.15	17.08	18.51
Gastric content	690.91	11.41	130.73	9.74	–
Bile	107.94	13.27	82.54	6.04	10.57
Urine	85.49	16.30	115.30	21.7	8.5

T: Tramadol; M1: O-desmethyltramadol; M2: N-desmethyltramadol; M3: N,N-didesmethyltramadol; M5: N,O-didesmethyltramadol.
Therapeutic range of blood tramadol concentrations: 0.1–0.8 mcg/ml.

Furthermore by full scan acquisition method (SCAN) it was detected the presence of hydroxytramadol characterized by mass spectrum: *m/z 58(100); 279(3); 135(3)* as well as was reported in Literature.[19]

Carbamazepine has been quantized in femoral blood (carbamazepine 3.2 mg/L). No other substances were found in any sample analyzed. Hair test resulted positive for tramadol and carbamazepine.

5. Discussion

Lethal overdose due only to tramadol is not common. More often the poisoning is due to tramadol in combination with other substances (alcohol, benzodiazepines, barbiturates, antidepressants, etc.).[9–17] In the case here illustrated, on the basis of our finding, it was possible to exclude that other toxic substances played a role, even marginal, in causing the death that was exclusively correlated to tramadol poisoning.

Therapeutic range of blood tramadol concentrations is about 0.1–0.8 mcg/ml[6]; toxic effects can be observed at blood concentrations above 1 mcg/ml (between 1 and 2 mcg/ml); the minimum lethal dose is 2 mcg/ml or higher.[6] In our case, the concentrations of tramadol found in viscera and biological liquids were the highest ever reported in the Literature in similar cases of pure tramadol poisoning. Table 2 illustrates the concentrations of tramadol (and some of its metabolites) reported by other Authors in fatalities caused mainly by this drug.[7,8,13,20–23]

Since the blood tramadol concentration of 61.83 mcg/ml (Table 1) is – at least – 80 times higher than therapeutic one (0.1–0.8 mcg/ml),[6] and 30-fold higher than that believed to be lethal, it can be presumed that the woman ingested a considerable amount of drugs. The question is how the woman survived until this high blood tramadol concentration was reached. Since the woman was a habitual consumer of tramadol it is possible hypothesize the develop of tolerance/abuse phenomena; In fact, although it is believed that tramadol has a low potential of abuse, especially on long-term use, tolerance, psychic and physical dependence may develop, with consequent tramadol abuse.[3] Thus, in this case, very high tramadol levels were necessary to cause death.

Table 2
Review of tramadol (mg/L – mg/kg) and its metabolites concentrations reported by other Authors in cases of tramadol overdose.

Reference	Sample	Tramadol	M1	M2
Lusthof and Zweipfenning (1998)	Blood	13		
Moore et al. (1999)				
	Blood	15.1		
	Urine	110.2		
	Liver	68.9		
	Kidney	37.5		
Musshoff and Madea (2001)				
	Blood	9.6		
	Urine	46.0		
	Liver	6.2		
	Kidney	46.1		
	Bile	3.1		
Loughrey et al. (2003)	Blood	3.7		
Clarkson et al. (2004)				
Case 1	Blood	1.6	0.44	0.09
Case 2	Blood	4.21	0.56	0.79
Case 3	Blood	7.9	3.1	1.3
Case 4	Blood	13.9	0.5	9.7
De Decker et al. (2008)				
	Blood	5.2		
	Liver	6.5		
	Kidney	4.5		
De Backer et al. (2010)				
Case 1	Blood	7.7	1.33	0.6
Case 2	Blood	48.3	2.43	10.09

M1: O-desmethyltramadol; M2: N-desmethyltramadol.

It is our opinion that in fatal tramadol poisonings a central role is played by M1. In fact, it has been reported that, compared to tramadol, the M1 metabolite shows the highest affinity to the human mu-opioid receptor[24] and should be considered responsible for the mu-opioid-derived analgesic effect.[24,25] Furthermore the affinity of the M1 metabolite to the mu-opioid receptor is 160–300 times greater than tramadol.[26]

After oral dose the mean peak plasma concentration of M1 occurs at three hours and remained stable for up to 10.5 h.[27] As suggested by Stamer et al. this lag period, necessary for the transfer of the hydrophilic active opioid compound across the blood–brain barrier, seems to be responsible for the delayed onset of central nervous system (CNS) toxicity.[27]

It is well known that tramadol is extensively metabolized after oral administration by a number of pathways. The major metabolic pathways are N- and O-demethylation and glucuronidation or sulfation in the liver. O-demethylation involves the polymorphic isozyme cytochrome P4502D6 (CYP2D6) and lead to O-desmethyltramadol (M1). N-demethylation occurs through cytochrome P4502B6 (CYP3A4) and lead to N-desmethyltramadol (M2).[28,29] It is also important to consider the variability of enzyme activities, for which CYP2D6 and CYP3A4 are subject to inhibition or induction by several drugs.

Blockade of M1 formation (for example, concomitant assumption of antidepressants → CYP2D6 inhibition) or a diversion of the metabolism of tramadol to M2 (for example, concomitant assumption of carbamazepine → CYP3A4 stimulation) could explain, even in cases of acute poisoning, a preferential formation of M2 over M1. In these instances, a longer time lapse is required to reach high levels of M1, especially in those subjects who developed tolerance.

Unlike the other occurrences,[8,13] in our case the woman was a habitual taker of carbamazepine. On the basis of our analytical findings and on blood carbamazepine concentration (3.2 mg/L) lower than the therapeutic range of 4–12 mg/L[6] – although it was likely to hypothesize that carbamazepine may have had some, if minor or even negligible, role – it was reasonable to exclude any acute toxic interaction. However, since it has been reported that the carbamazepine induces cytochrome CYP3A4 activity,[30,31] an increased tramadol N-demethylation could explain why M2 levels were greater than M1.

The pathogenic mechanism here hypothesized is in agreement with the study of Moore et al.[20] which proposed that the M1/M2 ratio could be useful as an indicator of the time lapse between ingestion of tramadol and death. These Authors suggested that a preferential formation of M1 over M2 (M1/M2 ratio > 1) is indicative of acute death, whereas preferential formation of M2 over M1 (M1/M2 ratio < 1) would suggest a longer time lapse after ingestion.[20]

6. Conclusions

In the present case, the toxicological findings (M1/M2 ratio < 1) are compatible with a prolonged depression of vital functions, as confirmed by histopathological results that highlighted a picture of inefficient heart pump activity with static blood flow.

For these reasons it is our opinion that the death was caused by a severe depression of fundamental functions (respiratory depression: bradypnea; bradycardia to cardiac arrest) as consequence of the acute tramadol intoxication and mediated by the M1 opioid-like activity.

Ethical approval

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Conflict of interest

None declared.

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